

A New Foliose-rooted Genus of Podostemaceae from Thailand with a Note on Root Evolution

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Based on comparative floral morphology, and supported by molecular phylogenetic data, the foliose-rooted *Thawatchaia trilobata* gen. et sp. nov. (subfamily Podostemoideae) is described from northern Thailand. Similarities and dissimilarities in vegetative and reproductive characters between *Thawatchaia* and related foliose-rooted Asian genera are discussed and the consequence of their bearing on generic recognition is noted. The marginal meristem, which gives rise to the foliose root, was probably established at the base of a lineage leading to *Thawatchaia*, *Hanseniella* and *Hydrobryum*. A key to the Asian foliose-rooted genera is provided.

Key words: *Hanseniella*, *Hydrobryum*, Podostemaceae, Thailand, *Thawatchaia trilobata* (gen. et sp. nov.)

The Podostemaceae are unusual aquatic angiosperms that occur on rocks in waterfalls and rapids. Vegetative plants grow submerged in swift-running water during the rainy season and become emerged and withered during the dry season when water level drops. Flowers generally open and seeds are set shortly after emergence. Adaptations to those extreme environmental conditions include variously flattened (nearly cylindrical, ribbon-shaped, or foliose), creeping and adhering (in some species floating) roots. The most specialized is the foliose root, as seen in Asian and African members of Podostemaceae.

There are four foliose-rooted genera in Asia, *Hanseniella*, *Hydrobryum*, part of *Diplobryum*, and part of *Zeylanidium*, all referred to subfamily Podostemoideae. The first three genera are distributed in Thailand and adjacent southeastern and eastern Asia, and the last occurs in Sri Lanka and

southern India. In all the genera the foliose root is lobed and dorsiventral to the rock surface with adventitious shoots (tufts of leaves) and flowers scattered on the dorsal surface and adhesive root hairs on the ventral surface. The genus *Hanseniella* is characterized by the 4-ranked, dimorphic (lobed and simple) bracts, ca. 10 ovules per ovary (i.e. ca. 5 per locule), and 8-ribbed capsule (Cusset 1992). *Hydrobryum* and *Diplobryum* are distinguished from *Hanseniella* by the 2-ranked, entire, uniform bracts, and 12-70 ovules per ovary (6-35 ovules per locule). *Hydrobryum* and *Diplobryum* differ in that *Hydrobryum* has 10-14 ribs on the capsule, and *Diplobryum* is characterized by the 20-ribbed capsule (Cusset 1972, 1992). Those diagnostic characters have recently been revealed by field and herbarium research of the Podostemaceae for the Flora of Thailand to be at least in part useless (M. Kato unpubl. data). An undescribed species of

Hanseniella has capsules with up to 16 ribs, and an undescribed species of *Hydrobryum* has fewer ovules than the rest of the genus and capsules with up to 20 ribs, as in *D. minutale*. A new definition is therefore needed to reflect accurately the systematic relationships. The southern Asian *Zeylanidium* differs from all other genera in that the capsule splits into 2 unequal valves.

During field research in northern Thailand,

we came across a foliose-rooted species. It is distinct from all foliose-rooted genera noted above, particularly in the bract morphology, and is described here as a new genus and species. This proposal is supported by a molecular phylogenetic analysis (Kita & Kato in press). The following is a key to the five genera with foliose roots in Asia, although the phylogenetic relationships of *Diplobryum* remain uncertain yet.

Key to Foliose-rooted Genera of Asia

1. Capsule splitting into 2 unequal valves
Zeylanidium (foliose-rooted species: *Z. olivaceum*, *Z. maheshwarii*)
1. Capsule splitting into 2 equal valves..... 2
2. Floral shoot erect or suberect; bracts 4-ranked, dimorphic, ventral ones (facing root surface) bilobed, dorsal ones simple *Hanseniella*
2. Floral shoot appressed or strongly oblique to root surface; bracts 2-ranked, uniform..... 3
3. Bract trilobed, lateral lobes acute, small lower bracts with small round lobes at base; capsule ribs 8-10 *Thawatchaia*
3. Bract simple, ovate; capsule ribs 12-20 4
4. Spathella splitting longitudinally; stalk of ovary straight or curved; capsule ribs 18-20.....
Diplobryum (foliose-rooted species: *D. minutale*, *D. vientianense*)
4. Spathella rupturing irregularly near apex or rarely splitting longitudinally (if spathella splitting longitudinally, then stalk of ovary always straight; ribs of capsule 12-17); capsule ribs 12-20 (if ribs 18-20, spathella rupturing irregularly)..... *Hydrobryum*

Thawatchaia trilobata M. Kato, Koi & Y. Kita,
gen. et sp. nov., Figs. 1, 2, 3A-C

Radices crustaceae, irregulariter lobatae; folia caespitosa, usque ad 3 mm longa; bracteae bifariae, usque ad 12, superne trilobatae, lobi acuti; flores valde obliqui vel appressi; tepala 2; stamina 2, infra medium connata; ovaria 2-locularia, ellipsoidea; ovula in quoque loculo 7-9; stigmata 2, aequalia, subintegra; capsula 8-10-costata. A *Hanseniella* et *Hydrobryum* bracteis bifaris, trilobatis differt.

Typus: Northern THAILAND. M. Kato, R. Imaichi, S. Koi & T. Wongprasert TL-1003 (holo BKF; iso TI), Mae Wang stream, north of Doi Inthanon Natl. Park, Chiang Mai, 450 m alt., 18° 38' N, 98° 43' E, December 24, 2003; fl. and fr.

Specimens examined: Northern THAILAND. M. Kato, S. Koi, Y. Kita & T. Wongprasert TL-419, M. Kato, C. Tsutsumi & T. Wongprasert TL-502, M. Kato, C. Tsutsumi & T. Wongprasert TL-503, Mae Klang waterfall, Doi Inthanon, Chiang Mai, 450 m alt., 18° 29' N, 98° 40'

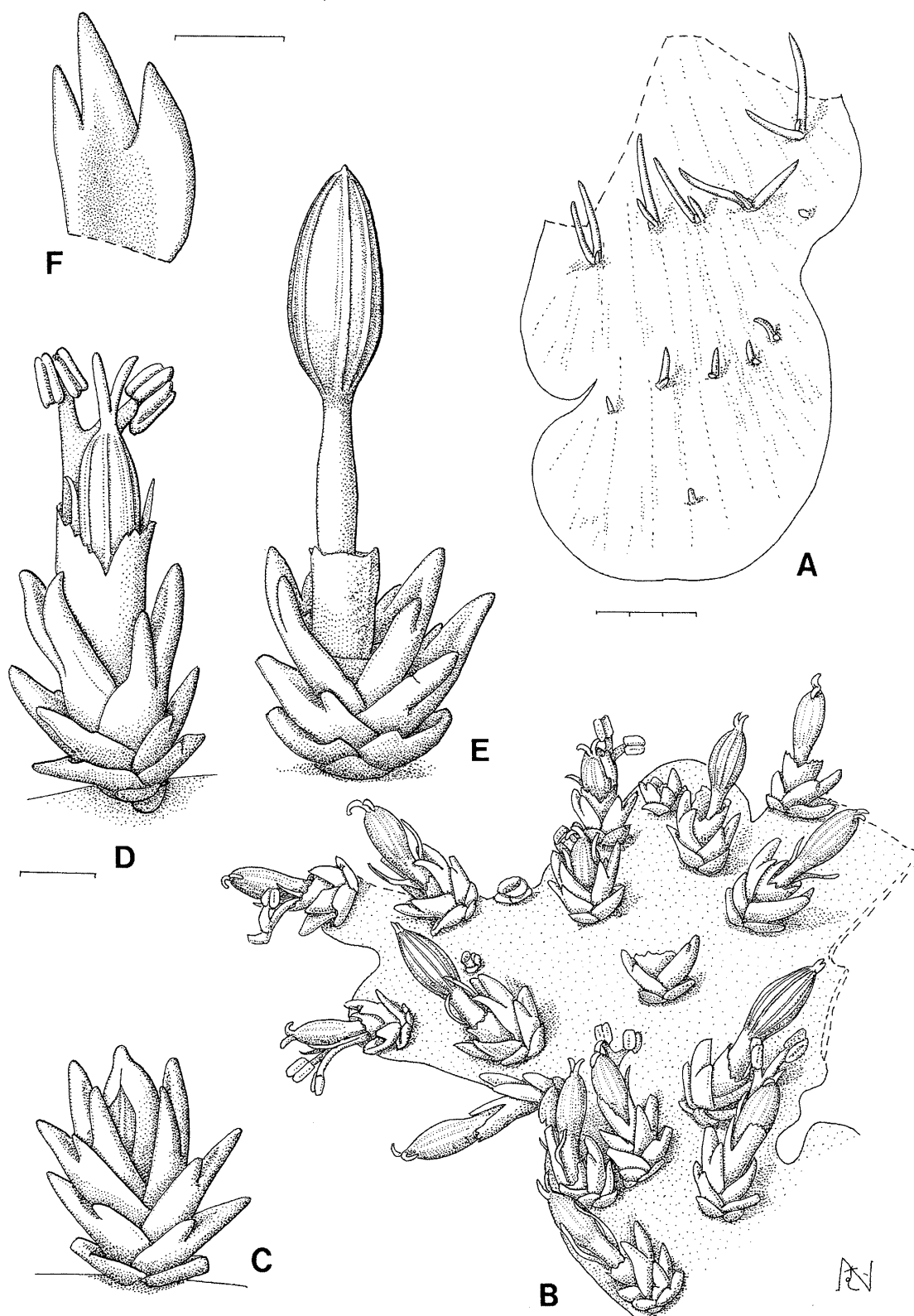


FIG. 1. *Thawatchaia trilobata*. Illustrations of Kato *et al.* TL-809 and TL-1003. A. Root with tufts of leaves. B. Root with flowers. C. Young flower. D. Flower. E. Young fruit. F. Bract. Scale bars = 3 mm in A, B; 1 mm in C-F.

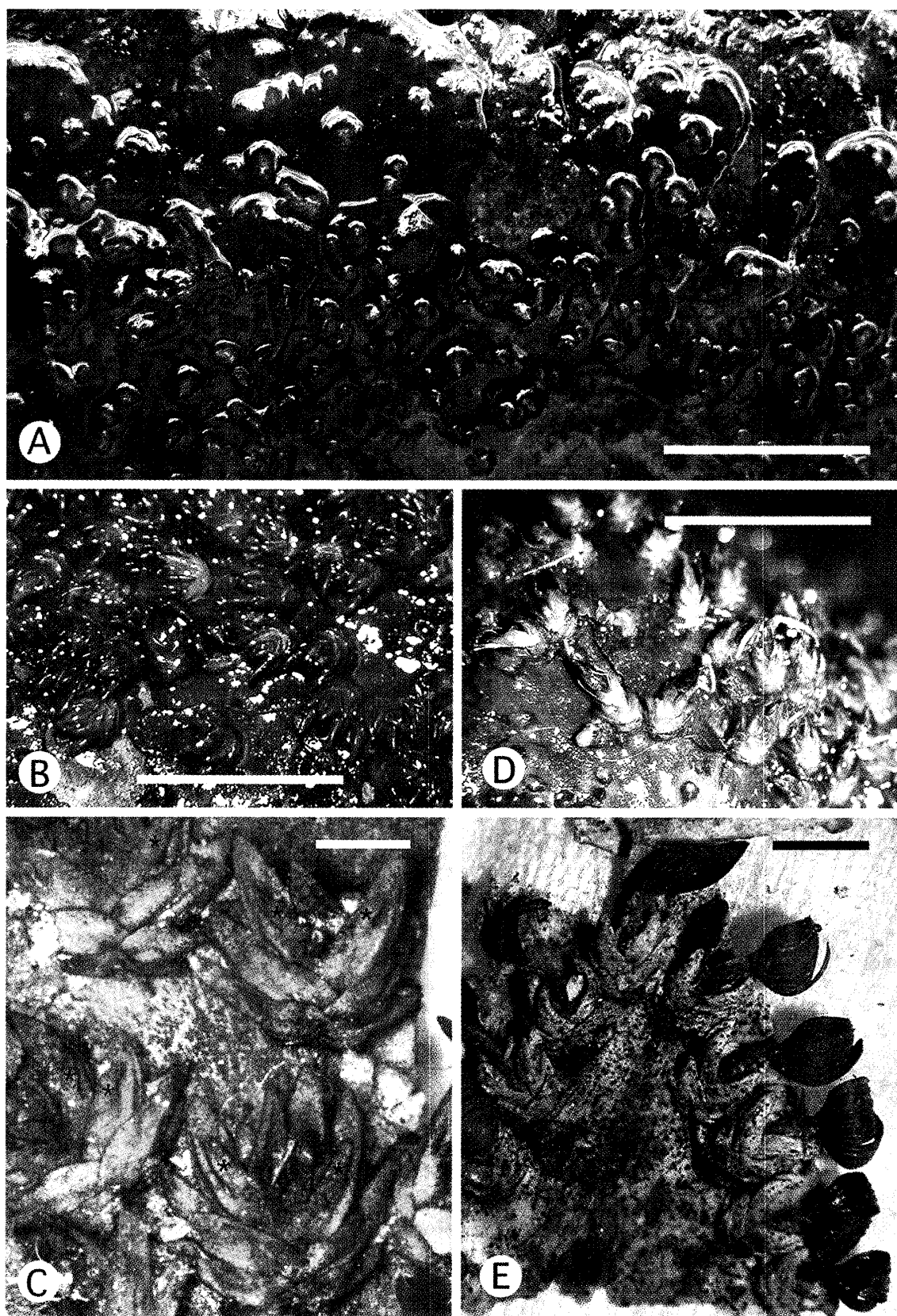


FIG. 2. Photographs of *Thawatchaia trilobata*. A. Foliose root with tufts of leaves on dorsal surface. Plant on rock in nature. Scale bar = 1 cm. B, C. Flower bud shoots. Asterisks indicate lateral bract lobes. C, dried specimen (Kato et al. TL-809). Scale bar = 1 cm in B; 1 mm in C. D. Flowers with red-purple ovaries at anthesis. Scale bar = 1 cm. E. Dehiscent capsules on withered root. Dried specimen (Kato et al. TL-809). Scale bar = 3 mm.

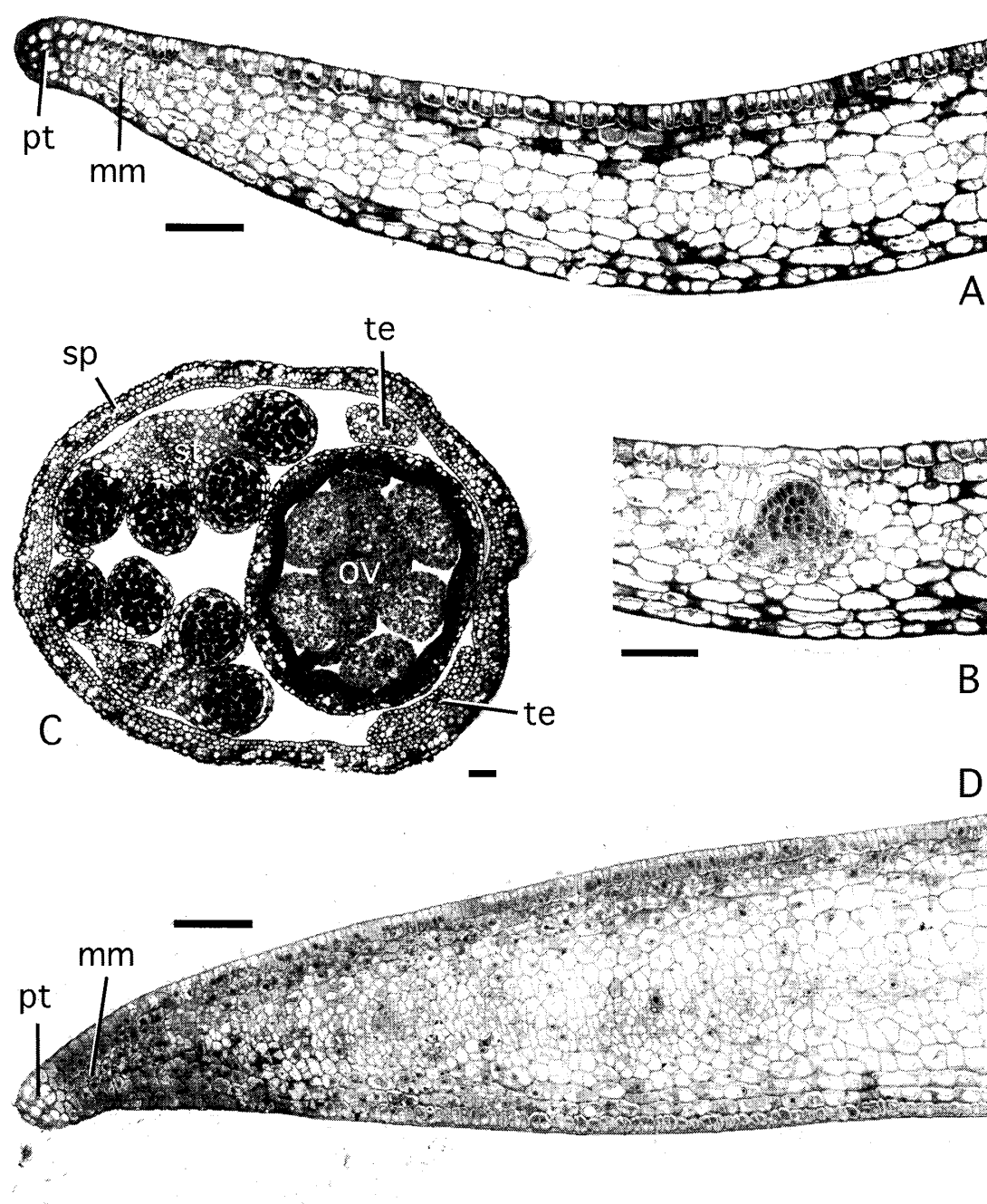


FIG. 3. *Thawatchaia trilobata*. Kato *et al.* TL-809. A. Longitudinal section of foliose root with marginal meristem and protective tissue. B. Longitudinal section of endogenous shoot primordium. C. Cross section of young flower. Note tip of curved stigma between stamens on left. D. Longitudinal section of root of *Hanseniella heterophylla*. Scale bars = 100 μ m. mm, marginal meristem; ov, ovary; pt, protective tissue; sp, spathe; st, stamen; te, tepal.

E, fl. bud, st. Dec., Mar.; M. Kato, R. Imaichi & T. Wongprasert TL-804, Wang Kwai waterfall near Forest Protection Station above Mae Klang waterfall, Doi Inthanon, Chiang Mai, 450 m alt., 18° 30' N, 98° 40' E, fl.

and fr. Mar.; M. Kato, R. Imaichi & T. Wongprasert TL-809, Mae Wang stream, north of Doi Inthanon Natl. Park, Chiang Mai, northern Thailand, 450 m alt., 18° 38' N, 98° 43' E, fl. and fr., Mar.

Roots foliose (crustaceous), irregularly lobed, ca. 0.3 mm thick, with tufts of leaves scattered on dorsal surface, patent, surrounded by root rim; leaves 2-5 per tuft, 2-ranked, linear, sheathed at base, terete apically, 1-3 mm long. Flowering shoots bearing solitary terminal flowers, scattered on dorsal surface of root, appressed or sometimes strongly oblique to root surface, rarely ascending, 2-3 mm long, flowers strongly oblique; bracts thick, 2-ranked, 4-6 per rank, upper ones trilobed or uppermost bract sometimes bilobed with ventral lateral lobe reduced, 1.5-3 mm long, lobes bluntly acute, ventral lobes smaller, lower bracts smaller than the upper, ca. 1 mm long with small round lobes at base; spathella thin, mucronate with mucrones ca. 0.3 mm long, ruptured irregularly near apex; stalk of ovary (peduncle) extruded from spathella, 2-2.5 mm long; tepals 2 on both sides of stamen, borne above base of ovary stalk, 2-3.5 mm long, reaching above middle of ovary, linear; stamens 2, connate near or above middle of filament, 3-5(-7) mm long, longer than ovary, anthers oblong-ellipsoid, ca. 1.2 mm long, withered shortly after rupturing of spathella; ovary 1, stalked (stalk of ovary ca. 2 mm long), ellipsoid, 2.5-3.5 mm long, locules 2, red-purple when fresh; stigmas 2, forked near base, equal, linear, entire, pointed, 0.6-1 mm long, withered shortly after extruding from spathella; ovules 7-9(-11) per locule, borne on marginal surface of septa or on entire septa surface except in small lower central area; capsule stalked (stalk 2-3 mm long), ellipsoid, flattened, 2-3 mm long, 1 mm wide, ribs 8-10.

Habitat: On rocks in waterfalls and rapids. *Thawatchaia trilobata* occurs with other podostemads in Wang Kwai waterfall and in Mae Wang stream.

Distribution: Thailand (Northern).

Taxonomic note: The genus is dedicated to Dr. Thawatchai Santisuk and Mr. Thawatchai Wongprasert. Dr. Thawatchai Santisuk guided us to Mae Klang waterfall, but we failed to find the plant on the first visit in 1999. He encouraged one of us (M.K.)

to investigate the Podostemaceae of Thailand for the Flora of Thailand. Mr. Thawatchai Wongprasert guided us to many different localities in Thailand, including Mae Klang waterfall and Mae Wang stream, to collect Podostemaceae. The Mae Wang (type locality) population was discovered by him.

In a molecular phylogenetic tree based on *matK* sequences, *Thawatchaia* is roughly as remote from *Hanseniella* (112 bp of 1530 bp sequenced) as from *Hydrobryum* including *Synstylis* [88 bp (mean) from nine species], compared with the interspecific differences among the species of *Hydrobryum* [50 bp (mean)] (Kita & Kato in press). The three genera and *Cladopus* form a monophyletic group, which is in turn sister to the *Zeylanidium-Polypleurum* group. This Asian group is close to the African and American groups of subfamily Podostemoideae, which are related to the other two subfamilies, Weddellinoideae and Tristichioideae (Kita & Kato 2001). Morphologically, *Thawatchaia* is similar to *Hanseniella* in some characters, while more similar to *Hydrobryum* in others (Table 1; see also Key). *Thawatchaia*, however, is distinct from *Hanseniella* in the flower shoot and flower insertions, number of ranks of bracts, bract uniformity and form, and number of ovules, and from *Hydrobryum* in the number of bracts, bract form, and number of ribs on the capsule (Table 1). Because of evidence from both molecular and morphological lines, we recognize *Thawatchaia* at the rank of genus. The genera are compared in more detail below.

Thawatchaia is similar to *Hanseniella*, *Hydrobryum*, part of *Diplobryum* and part of *Zeylanidium* in the foliose roots with tufts of leaves and flowers scattered on the dorsal surface, and the flower composed of a spathella (cover containing a flower bud), two linear tepals, two stamens (or rarely one) on a common filament (andropod), and a 2-locular ovary with two stigmas (Figs. 1, 2, 3C). *Thawatchaia* is most similar to *Hanseniella* among the foliose-rooted genera in the 10 or more lobed bracts,

TABLE 1. Comparison between *Thawatchaia* and Asian foliose-rooted genera of Podostemaceae. Data from Cusset (1972, 1992), Mathew & Satheesh (1997), Kato & Fukuoka (2002), Kato & Kita (2003), and M. Kato's unpublished results.

Characters	<i>Thawatchaia</i>	<i>Hanseniella</i>	<i>Hydrobryum</i>	<i>Diplobryum</i> *	<i>Zeylanidium</i> **
Flower shoot	appressed	erect or suberect	appressed	appressed	appressed
Flower	oblique	erect	appressed, rarely erect	erect	erect
No. of bract ranks	2	4	2	2	2
No. of bracts per rank	4-6	5-8	1-4	2-3	3-4
Bract uniformity	uniform	dimorphic	uniform	uniform	uniform
Bract form	trilobed	bilobed and simple	simple	simple	simple
Spathella break***	irr.	irr.	irr., rarely long.	long.	long.
Ovule no. per locule	7-9	4-6	4-29	22-35	45-50
Capsule valves	equal	equal	equal	equal	unequal
No. of capsule ribs	8-10	8, 12-16	12-20	18-20	8

* foliose-rooted species: *D. minutale* and *D. vientianense*.

** foliose-rooted species: *Z. olivaceum* and *Z. maheshwarii*.

*** irr., irregularly ruptured; long., longitudinally split.

less than 10 ovules per locule, and less than 10 ribs on the capsule wall. Lobed bracts also occur in *Cladopus*, which has subcylindrical or ribbon-like roots, and is sister to a clade of these genera and *Hydrobryum*. *Thawatchaia* and *Hanseniella* show further anatomical similarity in that the cortex of the root consists of three layers (Figs. 3A, D). The dorsal and ventral cortex is composed of elongate parenchyma cells, while the middle cortex is composed of short cells. In comparison, the root cortex is uniform in *Hydrobryum japonicum* (Ota *et al.* 2001) and *Zeylanidium olivaceum* and *Z. maheshwarii* (Hiyama *et al.* 2002).

Thawatchaia differs from *Hanseniella* in the flower shoots appressed or oblique to the root, the uniform and usually trilobed 2-ranked bracts, the thin spathella, and more than 5 ovules per locule. The 4-ranked bracts of *Hanseniella*, like those of *Willisia* (Uniyal & Mohan Ram 2001), are unusual in Asian Podostemoideae, including the clade of *Cladopus*, *Hanseniella*, *Hydrobryum* and *Thawatchaia*.

Thawatchaia is also similar to *Hydrobryum* in the flower shoots being oblique or appressed to the root surface, the bracts 2-ranked and uniform, and the spathella thin and ruptured irregularly near

the apex. It differs, however, in the thick trilobed bracts up to 6 per rank, the 7-9 ovules per locule (vs. usually more than 10 in *Hydrobryum* except in two undescribed species with 4 or 6 ovules), and the 8-10 capsule ribs.

Diplobryum comprises two foliose-rooted species and two other species with floating, cylindrical axes (roots or shoots) presumably branched from adhering roots (Cusset 1992, Kato & Fukuoka 2002), but perhaps the genus is not natural. The foliose-rooted species, *D. minutale* from southern Vietnam and *D. vientianense* from Laos, share the 4-6 simple bracts on 2 ranks (2 or 3 per rank), longitudinally split spathella, and capsules with about 20 ribs. Each of the characters, however, is seen in different species of *Hydrobryum* and a phylogenetic analysis is needed to make clear the systematics of *Hydrobryum* and *Diplobryum*. The phylogenetic relationship of *Diplobryum* and *Thawatchaia* is also uncertain, although the genera differ morphologically to a considerable degree (Table 1).

Zeylanidium is a southern Indian and Sri Lankan genus with two foliose-rooted species (*Z. olivaceum*, *Z. maheshwarii*) and a few others with subcylindrical or ribbon-like roots (Cusset 1992, Mathew & Satheesh 1997). A molecular phylo-

genetic study indicates that the genus is monophyletic and remote from the group of *Hanseniella*, *Hydrobryum* and *Thawatchaia* (Kita & Kato 2001, in press). *Zeylanidium*, like *Maferria*, is characterized by the capsule with two unequal valves, compared with the equal-valved capsules of the group of *Hanseniella*, *Hydrobryum* and *Thawatchaia* (for other characters, see Table 1).

Thawatchaia, like *Hanseniella*, is endemic to northern Thailand, in contrast to *Hydrobryum*, which is widely distributed from northern India eastward to Japan and southward to Thailand (Cusset 1992, Kato & Kita 2003). Taking into account these distributional data, the phylogeny with *Thawatchaia* and *Hanseniella* basal in the foliose-rooted group, and the occurrence of most species of *Hydrobryum* in northern Thailand, Thailand is likely to be a center of diversification of the Asian foliose-rooted Podostemaceae. This suggested biogeography is consistent with the high diversity of Podostemaceae - nearly 30 species - in Thailand, which is markedly more than in neighboring regions (M. Kato unpubl. data).

Note on root evolution: The foliose root is still an enigmatic organ. Compared to the absorbing and anchoring ordinary root, it is multifunctional, i.e. adhesive to the rock surface by root hairs; photosynthetic in place of reduced leaves, which may absorb carbon dioxide and oxygen and minerals; and reproductive by producing floral shoots. Phylogenetic analyses show that the foliose root of the group comprising *Hydrobryum*, *Hanseniella* and *Thawatchaia*, and that of *Zeylanidium*, are probably derived independently from a ribbon-like root similar to that in species of *Cladopus* and *Zeylanidium* (e.g. *Z. lichenoides*), respectively (Kita & Kato 2001, in press). A comparative study of seedlings also supports this independent evolution (Suzuki *et al.* 2002). In *Z. olivaceum* the secondary root forms endogenously in the hypocotyl, and a reduced primary shoot (plumule) forms between the cotyledons but is ephemeral. In *Hy. griffithii* and *Hy.*

micranthera the secondary root forms exogenously from the lateral side of the hypocotyl and no primary shoot is formed.

In the root of *Thawatchaia trilobata* and *Hanseniella heterophylla* there is a marginal meristem covered by a protective tissue or root cap (Figs. 3A, D). Tufts of leaves or reduced shoots are endogenous in the root. These characters are shared not only by *Hydrobryum japonicum* (Ota *et al.* 2001) (and perhaps *Diplobryum*) but also by *Zeylanidium olivaceum* and *Z. maheshwarii*, although the protective tissue is only slightly developed in *Z. maheshwarii* (Hiyama *et al.* 2002). Hiyama *et al.* (2002) hypothesized that the protective tissue of the *Zeylanidium* species appeared secondarily from the naked root meristem seen in *Z. lichenoides* and *Z. subulatum*.

The difference between the foliose and ribbon-like roots is due to a difference in the root meristem. In *Hydrobryum japonicum*, *Zeylanidium olivaceum* and *Z. maheshwarii*, the marginal meristem along the margin of the root lobe is uniform and determinate in the production of root tissues (Ota *et al.* 2001, Hiyama *et al.* 2002). It is likely that the other species of *Hydrobryum*, *Thawatchaia trilobata* and *Hanseniella heterophylla* have a similar root meristem. In comparison, the root of *Cladopus fukiensis* is narrow (ca. 0.6 mm wide) and that of *Z. lichenoides* and *Z. subulatum* is 1-2 mm wide. The apical meristem of their roots is indeterminate, as in typical angiosperm roots, but compressed as is the external morphology (Hiyama *et al.* 2002, Koi & Kato 2003). In a study of the developmental anatomy of *C. javanicus* with broadly ribbon-like (ca. 4 mm wide) roots, Koi & Kato (2003) showed that the root meristem consists of an apical meristem and marginal meristems on both sides of it. *Cladopus nymanii* is intermediate between *C. fukiensis* and *C. javanicus* in the root morphology and meristem structure. Koi & Kato (2003) inferred that reduction of the apical meristem or replacement by a marginal meristem was involved in the evolution of the foliose

root. The evolution may have occurred at the base of the lineage leading to *Thawatchaia*, *Hanseniella* and *Hydrobryum*. It is perhaps also the case with *Zeylanidium olivaceum* and *Z. maheshwarii*.

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